Parasitism of *Pharyngostomum cordatum* Metacercariae (Trematoda; Diplostomatidae) in Snakes, *Elaphe quadrivirgata* and *Rhabdophis tigrinus* (Reptilia; Colubridae)

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Abstract: Eighteen *Elaphe quadrivirgata* and 12 *Rhabdophis tigrinus* (Reptilia: Colubridae) captured in Okayama Prefecture, Japan, in the spring and summer of 1985 were examined for *Pharyngostomum cordatum* metacercariae (Trematoda: Diplostomatidae). The metacercariae were obtained from all the snakes examined together with plerocercoids of *Spirometra erinacei* (Cestoda: Diphyllbothriidae). The metacercaria burden in the host snakes was very great, being over one hundred in all the snakes examined and often reaching several thousand. This great parasite burden in snakes results from the biological concentration of parasites due to the predation on infected frogs by the snakes. The metacercariae were located in different tissues and organs, especially in the intercostal muscles and serosa. The metacercariae were sometimes enclosed by a cystlike membrane of connective tissue. Histopathological observation revealed that *P. cordatum* metacercariae were much less pathogenic to the host snakes than the *S. erinacei* plerocercoids which were found together with the metacercariae. In this situation, carnivorous final hosts can easily prey on the infected snakes, because the locomotive capacity of the snakes will be reduced by the parasitism of the cestode larvae. Accordingly, metacercariae of *P. cordatum* also will be transmitted to the final host together with *S. erinacei* plerocercoids. This phenomenon, that is, parasitism of *P. cordatum* metacercariae together with *S. erinacei* plerocercoids, can be considered one of the survival strategies of the parasite.

Key words: *Elaphe quadrivirgata*; *Rhabdophis tigrinus*; *Pharyngostomum cordatum*; Histopathology; Parasite ecology

Amphibians and reptiles are parasitized by many species of parasites and serve as not only their final hosts but also intermediate and/or paratenic hosts. Some of these parasites are of medical and veterinary importance. Two species, the diplostomid trematode *Pharyngostomum cordatum* and the pseudophyllidean cestode *Spirometra erinacei*, are well known as helminths transmitted through amphibians and reptiles to cats. We previously reported on the histopathological changes in the snake *Elaphe quadrivirgata* induced by *S. erinacei* plerocercoids and discussed their ecological meanings (Fukase et al., 1986a). In the present study, we examined the incidence of *P. cordatum* metacercariae and the histopathological changes induced by them in *E. quadrivirgata* and *Rhabdophis tigrinus*.

MATERIALS AND METHODS

Survey on *P. cordatum* metacercariae in snakes.—Eighteen adult *E. quadrivirgata* and 12 adult *R. tigrinus* (Reptilia; Colubridae),

more than 1 m long, were captured in Okayama Prefecture, Japan, in the spring and the summer of 1985, and examined for *P. cordatum* metacercariae.

Morphological observation of metacercariae.—Some of the metacercariae obtained were fixed in 70% (V/V) ethanol after being pressed between a slide glass and a cover slip (18 × 18 mm). They were then stained with van Cleave's combination hematoxylin, dehydrated in an ascending ethanol series, cleared in xylene, and mounted with canada balsam. These mounted specimens were observed by photomicroscopy, and 20 of them were measured.

Identification of parasites.—Some of the metacercariae were fed to two domestic cats. The cats were autopsied 40 days after infection, and adult flukes recovered from the small intestine were examined morphologically after being prepared by the same procedures as used for the metacercariae.

Histological observation of snake tissues.—For lightmicroscopical observation, blocks of snake tissues were dissected out and washed as quickly as possible in 0.025 M phosphate buffer, pH 7.4.

They were then fixed in buffered 10% (V/V) formalin. All the tissue blocks were routinely processed, embedded in paraffin, and sectioned at 4 μ m. Sections were stained, dehydrated in an ascending ethanol series, cleared in xylene, and mounted with canada balsam. The stains used were hematoxylin and eosin, Masson's trichrome, van Gieson, Watanabe's silver, alcian blue (pH 1.0), alcian blue (pH 2.5) and periodic acid-Schiff (PAS), toluidine blue (pH 7.0 and pH 2.5), and Giemsa (pH 7.0).

RESULTS

Detection of metacercariae.—Metacercariae were found in all the *E. quadrigata* and *R. tigrinus* examined, and all the snakes were also parasitized by *S. erinacei* plerocercoids. The number of metacercariae in one snake was very large, being over one hundred in all the snakes and often reaching several thousand. The metacercariae were located in different tissues and organs, most frequently in the intercostal muscles and serosa.

Two types of metacercariae were obtained: one enclosed by a cystlike membrane and the

other not enclosed. In the former type, one or occasionally two metacercariae were enveloped by the membrane.

Morphology of metacercariae.—The morphology of the metacercariae was as follows. The body was egg-shaped, about 0.5 mm in length and about 0.4 mm in maximum width at about the anterior 1/3 of body. The oral sucker, about 0.05 mm in diameter, was situated at the anterior end of body. The pharynx was about 0.06 mm in length and about 0.04 mm in width, located posteriorly to oral sucker, and followed by a straight esophagus. The esophagus bifurcated into two ceca in the middle between the pharynx and the ventral sucker (acetabulum). The ventral sucker, about 0.04 mm in diameter, was located near the center of the body. Posteriorly to the ventral sucker was located the holdfast organ (tribocytic organ), about 0.12 mm long and 0.09 mm wide, and having a sulcuslike structure.

Identification of parasites.—Two domestic cats were experimentally infected successfully and adult trematodes were recovered from the small intestine, especially from its upper part.

FIG. 1. *Pharyngostomum cordatum* metacercariae in the intercostal muscle of a snake, *Rhabdophis tigrinus*. Mc—metacercaria; Os—Os costale (costa).

FIG. 2. Metacercariae collected from a snake, *R. tigrinus*.

TABLE 1. Measurements (in mm) of *Pharyngostomum cordatum* metacercariae obtained from snakes.

	Length		Width	
	Range	Mean ± SD	Range	Mean ± SD
Body	0.445–0.580	0.517 ± 0.045	0.341–0.423	0.393 ± 0.031
Oral sucker	0.044–0.059	0.053 ± 0.006	0.049–0.064	0.055 ± 0.005
Pharynx	0.048–0.071	0.058 ± 0.007	0.031–0.049	0.040 ± 0.007
Ventral sucker (Acetabulum)	0.037–0.053	0.043 ± 0.005	0.035–0.052	0.046 ± 0.005
Tribocytic organ (Holdfast organ)	0.104–0.151	0.124 ± 0.014	0.069–0.106	0.085 ± 0.009

The trematodes were identified as *Pharyngostomum cordatum* by morphological examination on the adult flukes in addition to the metacercariae.

Histopathological findings.—Histopathological examination revealed that cell and tissue reactions were hardly induced in the affected snakes by the metacercariae, except for the formation of a thin membrane of connective tissue surrounding some of the metacercariae. No marked cell infiltration or proliferation of connective tissue was seen.

DISCUSSION

P. cordatum requires two kinds of intermediate hosts in addition to final hosts to complete the life cycle and often additionally utilizes paratenic hosts. The first intermediate host is freshwater snails of the genus *Polypylis* and so on, and the second intermediate and paratenic hosts are amphibians and reptiles, and sometimes birds and mammals. The animals recorded as the second intermediate and/or paratenic hosts of *P. cordatum* in Japan and

FIG. 3. Adult *P. cordatum* parasitic in the small intestine of an experimentally infected cat.

FIG. 4. Flattened adult *P. cordatum* specimen collected from the small intestine of an experimentally infected cat (van Cleave's combination hematoxylin stain).

FIG. 5. Histological picture around a metacercaria of *P. cordatum* in the muscle of a snake, *E. quadrivirgata* (Masson's trichrome stain, $\times 171$).

their localities are shown in Table 2.

The present study revealed that *E. quadrivirgata* and *R. tigrinus* from Okayama Prefecture were infected with a great number of *P. cordatum* metacercariae at a very high incidence rate. Although the incidence rate and worm burden will be different in different localities, snakes are thought to be generally parasitized by a larger number of the trematode larvae and at a higher frequency than frogs in a given locality. This is probably due to the fact that snakes prey on infected frogs and the metacercariae in the frogs are accumulated in snakes, a higher predator. We previously described a similar phenomenon in *S. erinacei* plerocercoids in snakes and considered it a kind of "biological concentration" (Fukase et al., 1986a), although this term is generally used regarding chemical substances. The accumulation of *P. cordatum* metacercariae in snakes is also a kind of "biological concentration".

Metacercariae of the strigeoid trematodes are generally known to form no cyst by themselves (Smyth and Halton, 1983), but meta-

cercariae of *P. cordatum* are sometimes reported to be enclosed by a membrane (Kurimoto, 1976; Uchida et al., 1977; Kajiyama et al., 1980; Uchida and Itagaki, 1980), as found in the present study. Histological examination has shown that this membrane is not of parasite origin (Ooi et al., 1984). The present study supported this view: the cystlike wall seemed to be of host origin, because it consisted of connective tissue and a single cystlike wall occasionally enclosed two metacercariae.

Histological reactions against the *P. cordatum* metacercariae were practically nil, so that pathogenicity of the metacercariae to snakes generally seemed to be slight, even in heavy infections.

We previously reported on the muscular lesions of snakes heavily infected with *S. erinacei* plerocercoids and discussed the increased possibility of the snakes captured by final host animals because of the reduced locomotive capacity of the infected snakes (Fukase et al., 1986a). *P. cordatum* metacercariae, however, do not seem to be so pathogenic that they reduce the locomotive capacity of snakes. All the snakes examined, however, were infected with both *P. cordatum* metacercariae and *S. erinacei* plerocercoids, probably due to the similarity in life cycle of these two helminth species. Accordingly, the snakes will be easily caught by carnivorous final hosts owing to the locomotive damage caused by *S. erinacei* plerocercoids. Thus, *P. cordatum* will be more successfully transmitted to the final hosts when the snakes are infected with *S. erinacei* plerocercoids in addition to the fluke species. This phenomenon seems to be one of the survival strategies of *P. cordatum*.

P. cordatum and *S. erinacei* utilize the same second intermediate, paratenic, and final hosts. The larvae of these two helminth species are very frequently found together in a snake, so that they will be transmitted together to a final host by predation. In fact, domestic cats are often infected with these two species of parasites (Oda et al., 1974; Iseki et al., 1974; Kifune and To, 1976; Maruhashi et al., 1977; Kajiyama et al., 1977; Uga et al., 1983; Sugano, 1985). Thus, the same host specificity of *P. cordatum* and *S. erinacei* may result from their similar subsistence in the course of evolution, although those two species of parasites belong to quite different taxonomical groups, the Trematoda and Cestoda, respectively. This phenomenon could be considered a kind of "adaptive concentration".

TABLE 2. Second intermediate and paratenic hosts of *Pharyngostomum cordatum* recorded in Japan.

Species	Locality	Authority
AMPHIBIA		
<i>Hyla japonica</i>	Aichi	Uchida and Itagaki (1980)
<i>Rana nigromaculata</i>	Aichi	Uchida and Itagaki (1980)
	Osaka	Kurimoto (1976)
	Yamaguchi	Kajiyama et al. (1980)
	Kagawa	Uchida et al. (1977)
<i>R. porosa</i>	Osaka	Kurimoto (1976)
<i>R. catesbeiana</i>	Aichi	Uchida and Itagaki (1980)
	Kagawa	Uchida et al. (1977)
<i>R. rugosa</i>	Aichi	Uchida and Itagaki (1980)
	Osaka	Kurimoto (1976)
	Kagawa	Uchida et al. (1977)
<i>R. limnocharis</i>	Kagawa	Uchida et al. (1977)
	Okinawa	Hasegawa (1984)
REPTILIA		
<i>Elaphe quadrivirgata</i>	Okayama	Fukase et al. (1986a, b)
	Kagawa	Uchida et al. (1977)
<i>Dinodon rufozonatus walli</i>	Okinawa (Iriomote-Is.)	Hasegawa and Iwatsuki (1984), Hasegawa (1985)
<i>Amphiesma pryeri</i>	Okinawa	Hasegawa (1985)
<i>Rhombophis tigrinus</i>	Aichi	Uchida and Itagaki (1980)
	Mie	Shiwaku et al. (1984)
	Okayama	Fukase et al. (1986a, b)
	Kagawa	Uchida et al. (1977)
<i>Trimeresurus okinavensis</i>	Okinawa	Hasegawa (1985)
MAMMALIA		
<i>Nyctereutes procyonoides</i>	Gifu	Ooi et al. (1984)

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要旨 シマヘビおよびヤマカガシにおける壺形吸虫のメタセルカリアの寄生

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1985年の春～夏に岡山県下で捕獲されたシマヘビ18個体、ヤマカガシ12個体について壺形吸虫のメタセルカリアの検索を行った。メタセルカリアは供試したヘビの全例から検出され、また、これらのヘビはすべて、同時にマンソン裂頭条虫の寄生も受けていた。ヘビ1個体あたりのメタセルカリア数はきわめて多く、全例100隻以上であり、数千隻に及ぶ例もしばしばみられた。このようなヘビにおける多数のメタセルカリアの寄生は、ヘビに捕食されたカエルに寄生していたメタセルカリアがヘビに感染したため、ある種の生物濃縮であると考えられた。メタセルカリアはヘビの様々な組織や器官から検出されたが、とくに肋間筋や漿膜組織に多数が認められた。なお、これらのメタセルカリアには、結合組織からなるシスト様の膜で包まれ

ているものもあった。組織学的観察の結果、壺形吸虫のメタセルカリアは、ヘビに対しての病害性をほとんど示さないように思われた。しかし、同時に寄生が認められたマンソン裂頭条虫のプレロセルコイドは病害性が強く、ヘビの運動能力を低下させると思われるので、これにより、終宿主である食肉目の動物にヘビが捕食される機会が増大することが推察される。したがって、壺形吸虫のメタセルカリアは、マンソン裂頭条虫のプレロセルコイドとともにヘビに寄生することにより、終宿主への感染の機会を増しているといえる。この現象は、壺形吸虫の適応戦略の1つであると考えられる。

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